Dr. Avery

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Summary of Dr. Avery's work on transformation prepared by Dr. Frank L. Horsfall, Dr. Rollin D. Hotchkiss and Dr. René J. Dubos. Includes bibliography of studies from Dr. Avery's laboratory on transformation.

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Oswald T. Avery discovered that nucleic acids of the desoxyribose type possess biologic specificity and are functionally important substances capable of orienting the biochemical activities and determining the heritable characters of microbial cells. This discovery was the most important result of a series of investigations, carried out in Avery's laboratory from 1928 to 1948, on the mechanism of experimental transformation of pneumococcal types. That such transformation could occur in vivo first was demonstrated by Griffith in 1928.

The culmination of the studies conducted by Avery and his coworkers came with the announcement, in 1944, that the fundamental constituent of the transforming agent of pneumococcus Type III is a polymerized desoxyribonucleic acid. The substance is extracted from heat-killed bacterial cells or in greater yield from cells caused to undergo lysis. Sodium citrate, which inhibits the inactivating effect of pneumococcal desoxyribonuclease, must be used in the latter procedure. After deproteinization and the removal of both capsular and somatic polysaccharides, the desoxyribomucleic acid is further purified by repeated precipitation with 0.2 volume of alcohol in the presence of calcium chloride. An exceedingly minute quantity of the purified material; i.e., 0.0015 microgram per ml., is capable in vitro of inducing transformation of competent cells placed under the proper cultural conditions.

Evidence that the transforming agent is a nucleic acid of the desoxyribose type was obtained by chemical, enzymatic, serologic and physico-chemical studies on highly purified material. The substance is soluble in water and gives colorless solutions which are highly viscous. Repeated precipitation with alcohol causes no loss of biologic activity.

Elementary analysis shows a phosphorus content of 8.5-9.0 per cent and a nitrogen-phosphorus ratio of 1.67-1.72. Ultraviolet absorption shows a high maximum at 2600 Å and a minimum at 2350 Å. Electrophoresis and ultracentrifugation yield in each case a single, sharp boundary with which the biologic activity migrates. In these respects, the material closely resembles authentic preparations of desoxyribonucleic acid of animal origin.

Crystalline trypsin, chymotrypsin and ribonuclease have no effect on either the physical properties or the biologic activity of the substance. On the other hand, crystalline desoxyribonuclease, in the presence of magnesium ions, causes marked loss in viscosity accompanied by complete and irreversible inactivation of transforming activity. As little as 0.001 microgram per ml. of the enzyme is effective. Citrate inhibits both the depolymerization of nucleic acid and the destruction of biologic activity by desoxyribonuclease. Reversible inactivation of the biologic activity is demonstrable with ascorbic acid and traces of cupric ion; reactivation is obtained on treatment with glutathione. Serologic procedures with antipneumococcal sera of high potency fail to reveal the presence of capsular or somatic polysaccharides or of pneumococcal protein, each of which would easily be demonstrated in such tests.

On hydrolysis of the purified agent, cytosine, adenine and thymine are found in amounts deviating from the equimolecular proportions which would be expected from the postulated structure of a tetranucleotide; uracil, a characteristic constituent of ribonucleic acids, is not present. Amino acids are obtained in so small an amount that not more than 0.2 per cent of protein could be present. Recent studies have shown that

glycine from the degradation of adenine is apparently the only amino acid present in an hydrolysate. The possibility that a specific protein or nucleoprotein, rather than the nucleic acid itself, is responsible for transforming activity seems, therefore, to have been excluded decisively.

Highly purified preparations of the transforming agent obtained from pneumococcus Type III made possible, for the first time, the induction of a predictable and permanent alteration in an heritable character of a living cell by means of a chemically defined substance of known nature. In other words, a specific mutation was induced as a result of a specific treatment. This is an achievement which has long eluded biologists.

The broad implications of the discovery of the nature of the transforming agent became apparent when it was demonstrated in Avery's laboratory that desoxyribonucleic acids, separated from a number of other types of pneumococci as well as various intratype mutants, possess predictable transforming activity relative to a specific cell character. Purified nucleic acids of the desoxyribose type prepared from Type II and Type VI pneumococci are closely similar in physical and chemical properties to that obtained from Type III and yet can be distinguished readily from one another by their specificity in the transforming system. Confirmatory evidence relative to other microbial species has been obtained in two laboratories. Boivin, et al. reported that a transforming agent similar in action and properties to that of pneumococci had been isolated from E. coli. Alexander and her associates showed that transformation of H. influenzae could be induced with

preparations of nucleic acids of the desoxyribose type.

Recent investigations of Hotchkiss have demonstrated that desoxyribomucleate factors isolated from penicillin or streptomycin resistant pneumococci are able to transform competent pneumococci and confer upon them corresponding drug resistance. Altogether, some sixteen transforming factors have been described for pneumococci; these comprise at least five series with different kinds of activities.

As a result of the studies of Avery and his associates, it is now apparent that certain polymerized desoxyribonucleic acids are concerned with the heredity of microbial cells in much the same fashion that genes are concerned with the heredity of higher organisms. It appears appropriate to think of the transforming agent of pneumococcus much as the geneticist thinks of the gene; as a self-duplicating agent which initiates a series of reactions resulting in the elaboration of a specific substance. In the case most thoroughly studied, the final product is the capsular polysaccharide of pneumococcus. It seems highly significant that a means has been developed for the isolation of material possessing the fundamental properties of a genetic determinant, in the form of a purified, stable nucleic acid suitable for biologic and chemical investigation.

The impact of these findings upon prevailing concepts in related fields is not difficult to trace. Investigators in cytology, genetics and virology, aware that desoxyribonucleic acid is a prominent constituent of nuclei, chromosomes and viruses, were faced with the proposition that this constituent is the one functionally operative in transmitting the manifold biologic capacities and potentialities of the germ

plasm. This, in turn, indicated to biochemists that mucleic acids are capable of inmumerable variations in composition and structure. Both of these concepts have received considerable support and it has become increasingly evident that they play an important part in orienting investigations in the several fields concerned with cellular development and differentiation.

This summary of Dr. Avery's work on transformation was prepared by Dr. Frank L. Horsfall, Dr. Rollin D. Hotchkiss, and Dr. René J. Dubos.

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Herbert J. Gasser

BIBLIOGRAPHY

I. Studies from Avery's laboratory on transformation:

1. Dawson, M. H., The transformation of pneumococcal types. I. The conversion of R forms of pneumococcus into S forms of the homologous type. II. The interconvertibility of type-specific S pneumococci. J. Exp. Med., 1930, 51: 99-122 and 123-147.

2. Alloway, J. L., The transformation in vitro of R pneumococci into S forms of different specific types by the use of filtered pneumococcus extracts. J. Exp. Med., 1932, 55: 91-99.

3. Alloway, J. L., Further observations on the use of pneumococcus extracts in effecting transformation of type in vitro. J. Exp. Med., 1933, 57: 265-278.

4. Avery, O. T., MacLeod, C. M., and McCarty, M., Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus Type III. J. Exp. Med., 1944, 79: 137-158.

5. McCarty, M., Reversible inactivation of the substance inducing transformation of pneumococcal types. J. Exp. Med., 1945, 81: 501-514.

6. McCarty, M., and Avery, O. T., Studies on the chemical nature of the substance inducing transformation of pneumococcal types. II. Effect of desoxyribonuclease on the biological activity of the

transforming substance. III. An improved method for the isolation of the transforming substance and its application to pneumococcus Types II, III, and VI. J. Exp. Med., 1946, 83: 89-96 and 97-104.

7. McCarty, M., Chemical nature and biological specificity of the substance inducing transformation of pneumococcal types. Bact. Rev., 1946, 10: 63-71.

8. McCarty, M., Taylor, H. E., and Avery, O. T., Biochemical studies of environmental factors essential in transformation of pneumococcal types. Cold Spring Harbor Symposia on Quantitative Biology, 1946, 11: 177-183.

9. Taylor, H. E., Additive effects of certain transforming agents from some variants of pneumococcus. J. Exp. Med., 1949, 89: 399-424.

10. Hotchkiss, R. D., Études chimiques sur le facteur transformant du pneumocoque, 8e Colloque International du Centre National de la Recherche Scientifique, Unités biologiques douées de contimuité génétique, Paris, June 25 - July 3, 1948, Paris, Centre National de la Recherche Scientifique, 1949, pp. 57-65.

11. Taylor, H. E., Nouvelles transformations induites spécifiquement chez le pneumocoque, 8e Colloque International du Centre National de la Recherche Scientifique, Unités biologiques douées de contimuité génétique, Paris, June 25 - July 3, 1948, Paris, Centre National de la Recherche Scientifique, 1949, pp. 45-55.

II. Continued studies on transformation:

1. Hotchkiss, R. D., The biological nature of the bacterial transforming factors. Chemistry and Physiology of the Nucleus, V. T. Bowen, editor. Brookhaven Conference, 1951, Academic Press, New York, 1952.

2. Hotchkiss, R. D., The chemical nature of the pneumococcal transforming factor. In preparation.

III. Collateral studies on transformation:

1. Griffith, F., The significance of pneumococcal types. J. Hyg., 1928, 27: 113-159.

2. Boivin, A., Delaunay, A., Vendrely, R., and Lehoult, Y., L'acide thymomucléique polymérisé, principe paraissant susceptible de déterminer la specificité sérologique et l'équipment enzymatique des bactéries. Signification pour la biochimie de l'hérédité. Experentia, 1945, 1: 334-335.

3. McCarty, M., Purification and properties of desoxyribonuclease isolated from beef pancreas. J. Gen. Physiol., 1946, 29: 123-139.

4. MacLeod, C. M., and Krauss, M. R., Stepwise intratype transformation of pneumococcus from R to S by way of variant intermediate in capsular polysaccharide production. J. Exp. Med., 1947, 86: 439-453.

5. Taylor, H. E., Biological significance of the transforming principles of pneumococcus. Pubb. Stazione Zool. di Napoli, 1950, 22: 65-77.

6. Alexander, H. E., and Leidy, G., Determination of inherited strains of H. influenzae by desoxyribomucleic acid fractions isolated from type-specific cells. J. Exp. Med., 1951, 93: 345-359.

ASSOCIATES OF AVERY WHO INVESTIGATED TRANSFORMATION:

	Worked with Avery
Dawson, Martin H.:	From July 1926 to June 1929.
Alloway, James L.:	From July 1930 to June 1932.
MacLeod, Colin M.:	From July 1937 to July 1941.
McCarty, Maclyn:	From September 1941 to July 1945.
Taylor, Harriett E.:	From September 1945 to October 1947.
Hotchkiss, Rollin D.:	From July 1935 to July 1942, and From July 1946 to July 1948.